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PHARMACOGNOSTIC ANALYSIS AND PHYTOCHEMICAL SCREENING OF METHANOLIC EXTRACT OF IPOMOEA BATATA LEAVES

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ABSTRACT

The history of plants being used for various medicinal purposes is been recorded since olden days. According to WHO, for 80% of the world especially for common people including in developed countries herbal medicine is the one for which they have ready access. Evaluation of plant medicines has to be based on conceptual and theoretical groundwork of the traditional system of medicine, and should include utility of the progression in scientific knowledge. Several studies are needed to be conducted to develop the phytochemical and pharmacognostic profile of plant medicine based on which its pharmacological screening of plant extracts can be designed to develop new plant medicines or lead molecules. Hence the objective of the present study was to assess the pharmacognostic parameters of ipomoea batatas leaves to determine its quality and then followed by assessing the organoleptic and phytochemical nature of the methanolic extracts of Ipomoea batatas leaves. The selected plant part was dried, powdered and subjected to cold extraction i.e., maceration using methanol as solvent. The extract was dried for evaporation of solvent and then subjected to organoleptic and phytochemical studies. The phytochemical studies indicate that the Ipomoea batatas methanolic extract showed presence of alkaloids, carbohydrates, flavonoids, triterpenoids, steroids and glycosides. The results of pharmacognostic studies revealed the physicochemical parameters of ipomoea batatas leaves.

KEYWORDS: Methanolic extract, Ipomoea batatas leaves, phytochemical analysis, and pharmacognostic studies.

Introduction:
Sweet potato is one of the oldest vegetable known to mankind. It is used for consumption for centuries. Its history dates back to 750 B.C. in Peruvian records. Sweet potato is a large, starchy, tuberous root vegetable. Each and every part of the sweet potato, especially the tuber is beneficial for society. This plant is dicotyledenous and belongs to the family Convolvulaceae is scientifically known as Ipomoea batatas L. Several species of this plant have been commonly used in religious rituals and also for ornamental and medicinal purposes. Due to its several neutraceutical components and carotenoids it is considered as a health food. Sweet potato contains magnesium, the key mineral for de-stressing and good mood. It also promotes artery, bone, muscle, and nerve health. Sweet potato varieties may be 'firm' or 'soft'. [1]

The Decoctions of the Ipomoea batatas leaves can be used as an aphrodisiac, astringent, demulcent, laxative, energizer, bactericide and fungicidal agent. Sweet potato has been found to be beneficial in treating various health problems like asthma, bugbites, burns, catarrh, convalescence,diarrhea, fever, nausea, splenosis, stomach distress, and whitlows (an infection of tip of finger) and enhances immunity. In region of Kagawa, Japan, a variety of white sweet potato has been eaten raw to treat anemia, hypertension and diabetes.[2]

Herbal medicines appear to be quite effective in treating various clinical disorders furthermore; these herbal drugs are essentially safe. As stated above Ipomoea batatas is rich in nutritional value and also been used for various medicinal purposes there is need for exploring its phytochemical constituents, pharmacognostic studies to develop valid scientific data to further assess its pharmacological activity. Hence the present study was carried out.

Materials and methods:
Plant Materials: Leaves of Ipomoea batatas were collected from local region of Tirupati and was authenticated by Head of the Department, Department of Botany, S.V Arts College, Tirupati.

Chemicals: Methanol, alcohol, α- naphthol, concentrated sulphuric acid,dilute hydrochloric acid, glacial acetic acid, acetone, choloroform, and Distilled water used were analytical grade.

Pharmacognostic Analysis:
The pharmacognotic analysis of dried sample of ipomoea batata leaves were carried out as follows.

Physicochemical parameters:
Physiochemical values like the percentage of ash values and extractive values etc., were determined according to the official methods 3,4 and as per WHO guidelines on quality control methods for medicinal plant materials5,6.

Water soluble extractive value of Ipomoea batatas leaves:
About 5 g of powdered Ipomoea batatas leaves was added to 50 ml of water at 80°C in a stoppered flask. It was shaken well and allowed to stand for 10 minutes. It is then cooled to 15°C followed by addition of 2g of kieselghur and filtered. 5 ml of filtrate was transferred to tarred evaporating basins and evaporated on a water bath and the residue was weighed. The percentage of water soluble extractive was calculated with reference to one gram of dried sample of plant extact.[20]

Alcohol soluble extractive value of Ipomoea batatas leaves:
About 5 g of powdered Ipomoea batatas leaves was macerated with 100 ml of 90% ethanol in a closed flask for 24 h, shaking frequently during 6 h and allowed to stand for 18 h. It was filtered immediately taking precaution against loss of alcohol and 25 ml of filtrate was evaporated to dryness in a tarred flat bottomed shallow dish and dried at 105°C and weighed. The percentage of alcohol soluble extractive was calculated with reference to one gram of dried sample of Ipomoea batata leaves.[20]

Loss on drying of Ipomoea batatas leaves:
About 1 gm of powdered Ipomoea batatas leaves was transferred into a petridish plates and the content was distributed evenly to a depth not exceeding 10 mm. The loaded plate was heated at 105°C in hot air oven for 1 hr and then cooled in desiccator, loss in weight was recorded as moisture content or loss on drying. The moisture content percentage of the sample was calculated.

$$\text{Formula:} \quad \frac{w'_2 - w_3}{w_2 - w_1\,(100-H)} \times 100$$

W_1 = Empty Petri-dish weigh,

w_2 = Petri-dish + sample weight,

w_3=Petri-dish + sample weight after oven,

w'_3 = Weight after desiccate,

H = loss on drying

Total ash value of Ipomoea batatas leaves:
The total ash was determined by incinerating 2 g of powdered Ipomoea batatas leaves in a tarred silica crucible which was previously ignited and cooled before weighing. The drug was incinerated by gradually increasing the heat in a muffle furnace at 450°C for 4 hrs. Till the constant weight was obtained ignition was repeated . After complete incineration, it was cooled in a desiccator. Then the percentage of the total ash with reference to per gram of dried sample of Ipomoea batatas leaves was calculated.[20]

$$\text{Formula:} \quad \frac{w'_3 - w_1}{w_2 - w_1\,(100-H)} \times 100$$

w_1 = Empty crucible weight,

w_2 = Crucible + sample weight,

w_3 = Crucible + sample weight after burning,

w'_3 = Weight after desiccate,

H = Loss on drying

Acid – insoluble ash of Ipomoea batatas leaves:
The ash of powdered Ipomoea batatas leaves was washed from the crucible into 100 ml beaker using 25 ml of 2 N HCl. Then boiled for 5 min over a Bunsen burner and filtered through an ashless filter paper (Whatman No: 42).Using hot water the residue was washed twice, ignited to ash, cooled in desiccator and weighed. The residue was weighed and the acid insoluble ash of the drug was calculated with reference to the dried sample of Ipomoea batatas leaves.[20]

$$\text{Formula:} \quad \frac{w'_4 - w_1}{w_2 - w_1\,(100-H)} \times 100$$

w_1 = Empty crucible weight,

w_2 = Crucible + sample weight

w_3 = Crucible + sample weight after burning,

w_4 = Burn filter paper + crucible weight,

w'_4 = Weight after desiccate,

H = Loss on drying

Foreign matter in Ipomoea batatas leaves:
About 250 g of powdered Ipomoea batatas leaves was weighed. It was spread in a thin layer and foreign matter was sorted into groups with the help of a sieve. Sifted the remainder of the sample through a No. 250 sieve. 0.05 g of sorted foreign matter was weighed. Calculated the content of each group in grams per 100 g of air-dried sample.[5]

Preparation of extracts:
To prepare the methanolic extract, 150 g of Ipomoea batatas leaves was collected, air dried and reduced to powder. It was macerated with methanol and was allowed to stand for 72 hrs at room temperature and then filtered. The filtrate was then evaporated under reduced pressure and dried using a rotary evaporator at 55°C. Dried extract was stored in labeled sterile screw capped bottles at 5°C in the refrigerator.[9] The prepared extracts were used for Organoleptic characterization, and screening of phytochemical parameters.

Phytochemical analysis:
Preliminary phytochemical screening:
Plants are considered as bioreactors or biosynthetic laboratories as they synthesize wide range secondary metabolites which are therapeutically important. Thus, to establish a chemical profile of a crude drug for its proper evaluation a systematic preliminary phytochemical screening of plant material is essential for identifying plant constituents. Phytochemical screening of the methanolic extract of Ipomoea batatas leaves was carried as per systematic methods.[7,8].

Test for Carbohydrates:
Molisch's test: To 2 ml of methanolic extract, two drops of alcoholic solution of α- naphthol were added. The mixture was shaken well and few drops of concentrated sulphuric acid was added slowly along the sides of test tube.[10]

Fehling's test: In a test tube 2 ml of methanolic extract was taken and equal volumes of Fehling A & Fehling B solutions were added and placed it in a boiling water bath for few minutes.[10]

Benedict's test: To 0.5 ml of methanolic extract, 0.5 ml of Benedict"s reagent was added. The mixture was heated for 2 minutes on a boiling water bath.[10]

Barfoed's test: To 2 ml of methanolic extract about 2-3 ml of Barfoed's reagent. Mixed it well and boiled it for one minute in the water bath and allowed to stand for a few minutes.[10]

Test for Proteins:
Biuret test: 2 ml of methanolic extract was treated with 1 drop of 2% copper sulphate solution. To this 1 ml of ethanol (95%) was added and followed by addition of excess of potassium hydroxide pellets.[11]

Millon's test: To 2 ml of methanolic extract few drops of Millon's reagent was added.[12]

Test for Aminoacids:
The methanolic extract is dissolved in 10 ml of distilled water and filtered through Whatmann No. 1 filter paper and the filtrate is subjected to test for Amino acids. [10]

Ninhydrin test: Two drops of ninhydrin solution (10 mg of ninhydrin in 200 ml of acetone) was added to 2 ml of methanolic extract. [13]

Test for Flavonoids:
Shinoda test: Few magnesium chips were added to 2 ml of the methanolic extract and then 2 drops of dilute hydrochloric acid was added and warmed.[14]

Alkaline test: The methanolic extract was treated with 10% ammonium hydroxide solution.[10]

Test for PolyPhenols:
Ferric chloride test: The methanolic extract was dissolved in 5 ml of distilled water. To this few drops of neutral 5% ferric chloride solution was added.[15]

Lead acetate test: The methanolic extract was dissolved in of distilled water and to this 3 ml of 10% lead acetate solution was added.[10]

Bromine water test: Three drops of bromine water were added to 2 ml of methanolic extract.[14]

Test for Glycosides:
Borntrager's test: To 2 ml of methanolic extract, 3 ml of choloroform was added and shaken, then choloroform layer is separated and 10% ammomia solution was added to it.[16]

Keller-killiani test: 0.5 ml of methanolic extract was dissolved in 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was then underlayed with 1ml of concentrated sulphuric acid.[14]

Test for Alkaloids:
Dragendorff's: To 2 ml of methanolic extract in test tube, 1 ml of dragendoff's reagent was added drop by drop.[14]

Mayer's test: To a few ml of methanolic extract, two drops of Mayer's reagent was added along the sides of test tube.[16]

Wagner's test: A few drops of Wagner's reagent was added to few ml of methanolic extract along the sides of test tube.[17]

Hager's test: To 2 ml of methanolic extract, two drops of Hager's reagent was added.[18]

Test for Steroids-Terpenoids:
Salkowski Reaction: To 2 ml of methanolic extract in test tube, 3 drops of concentrated sulphuric acid was added to form a lower layer.[14]

Liebermann-Buchard reaction: The methanolic extract was dissolved in 2 ml acetic anhydride. To this, 2 drops of concentrated sulphuric acid was added slowly along the sides of the test tube.[19]

Results and discussion:
Pharmacognostic Analysis:
The pharmacognostic studies revealed the physicochemical parameters of the selected plant powder like foreign organic matter, alcohol soluble extractive, water soluble extractive, pH, Loss on drying, ash content and acid insoluble ash as shown in table 1 - which might help us in assessing its quality.

Table 1: Physicochemical parameters of powdered Ipomoea batatas leaves.

Parameter	IE
Water soluble extractive	8.43%
Alcohol soluble extractive	12.29%
Loss on drying	4.32%
Ash content	5.29%
Acid insoluble ash	0.63%
Foreign organic matter	1.54%
pH 1%w/v	6.36

Organoleptic characters:
The organoleptic characters of the methanolic extract of the plant are shown in table – 2.

Table 2: Organoleptic characters of methanolic extracts of Ipomoea batatas leaves.

Parameter	IE
Colour	green
Odour	characteristic
Taste	characteristic
Physical appearance	Free flowing powder

Phytochemical Analysis:

The phytochemical analysis showed presence of carbohydrates, flavonoids, glycosides, alkaloids, steroids and triterpenoids as shown in table – 3. The presence of above constituents in the plant extracts alone or in combination might be responsible to exhibit its pharmacological activity. Hence, the further pharmacological screening and application of modern scientific technology might help us to derive therapeutically active compounds.

Table 3: PHYTOCHEMICAL TEST OF METHANOLIC EXTRACTS OF IPOMOEA BATATAS LEAVES.

Test	Result
Test for Carbohydrates:	
a) Molisch's test	+
b) Fehling's test	+
c) Benedict's test	+
d) Barfoed's test	+
Test for Proteins:	
a) Biuret test	_
b) Millon's test	_
Test for Aminoacids:	
a) Ninhydrin test	_
Test for Flavonoids:	
a) Shinoda test	+
b) Alkaline test	+
Test for Polyphenols:	
a) Ferric chloride test	_
b) Lead acetate test	_
c) Bromine water test	_
Test for Glycosides:	
a) Borntrager's test	+
b) Keller-killiani test	+
Test for Alkaloids:	
a) Dragendorff's	+
b) Mayer's test	+
c) Wagner's test	+
d) Hager's test	+
Test for Steroids-Terpenoids:	
a) Salkowski Reaction	+
b) Liebermann-Buchard reaction	+

*(-): Absent, (+): Present.

Summary and conclusion:

Current trend in research is focussing more on deriving potent natural drugs from plants and developing the standardization techniques of herbal medicine to enhance its global acceptance. Synthetic drugs available in market are found to pose side effects so to overcome this problem more natural compounds with potent therapeutic value with different activities like antimicrobial, anti inflammatory, hepatoproctive and immunomodulatory etc., should be developed. Hence, extensive research in plant medicine should be carried out to establish its properties, efficacy and safety. In the present study, the selected part of the plant was collected, dried and subjected to size reduction to get uniform coarse powder. The powdered material of plant was then subjected to pharmacognostic studies to determine its physicochemical parameters. The pharmacognostic studies revealed the important parameters of the plant extract like foreign organic matter, alcohol soluble extractive, water soluble extractive, pH, Loss on drying, ash content and acid insoluble ash. Then the powdered plant material was subjected to cold extraction i.e maceration with methanol at room temperature. The methanolic extract of Ipomoea batatas leaves were dried at reduced temperature using rotary evaporator. The dried extract was then subjected to organoleptic and phytochemical analysis. The methanolic extract of Ipomoea batatas leaves showed presence of alkaloids, carbohydrates, flavonoids, triterpenoids and steroids and glycosides. Hence, the present study confirms the presence of diverse group of compounds and might prove as rich source of therapeutic value. Extensive study might provide medicinally active compounds.

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